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Folic acid antagonist use before and during pregnancy and risk for selected birth defects

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Abstract

Background: Maternal folic acid (FA) intake before and during early pregnancy reduces the risk for neural tube defects (NTDs); evidence suggests it may also reduce the risk for oral clefts, urinary defects, and cardiac defects. We sought to re-examine the use of drugs, which affect folate metabolism, dihydrofolate reductase inhibiting (DHFRI) medications, and anti-epileptic drugs (AEDs), in data collected in the post-FA fortification era (1998+) in the Slone Birth Defects Study.

Methods: We assessed maternal DHFRI and AED use and risk for NTDs, oral clefts, and urinary and cardiac defects. We estimated odds ratios (ORs) and 95% confidence intervals (CIs) using logistic regression. We assessed daily average FA intake of 400 mcg as a potential effect modifier.

Results: We analyzed data from 10,209 control and 9,625 case mothers. Among controls, the prevalence of exposure to DHFRI medications was 0.3% and to AEDs was 0.5%. Maternal use of AEDs was associated with increased risks for NTDs (OR: 3.4; 95% CI: 1.5, 7.5), oral clefts (OR: 2.3; 95% CI: 1.3, 4.0), urinary defects (OR: 1.6; 95% CI: 1.0, 2.7), and cardiac defects (OR: 1.6; 95% CI: 1.1, 2.3); similar or further increased risks were found among those with FA intake 400 mcg per day. DHFRI use was rare and relative risk estimates were imprecise and consistent with the null.

Conclusions: Similar to our previous analyses, we observed associations between AED use and these defects. For DHFRI exposure, we found no evidence for increased risk of these defects. Though statistical power to examine FA effect modification was low, we found no evidence of further protection among those with FA intake 400 mcg, with some associations somewhat stronger in this group.

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1 | INTRODUCTION

Maternal folic acid intake before and during early pregnancy reduces the risk of neural tube defects (NTDs) and some studies suggest that it may also reduce the risk of oral clefts, urinary defects, and cardiac defects (Botto, Khoury, Mulinare, & Erickson, 1996; Canfield et al., 2005; Czeizel, 1996; Czeizel, Tóth, & Rockenbauer, 1996; Ingrid Goh, Bollano, Einarson, & Koren, 2006; Shaw, O'Malley, Wasserman, Tolarova, & Lammer, 1995; M. M. Werler, Hayes, Louik, Shapiro, & Mitchell, 1999; Wilcox et al., 2007). Folic acid antagonists, medications that affect folate metabolism, have the potential to increase the risk for folate-sensitive birth defects. Previous analyses have identified two major groups of these drugs: dihydrofolate reductase inhibitors (DHFRIs), and anti-epileptic drugs (AEDs).

Although the exact mechanisms are not known, data suggest that DHFRIs and AEDs likely affect folic acid through different pathways. Dietary folate and synthetic folic acid are converted by the enzyme dihydrofolate reductase into tetrahydrofolate, which is then used in methylation pathways and for nucleotide synthesis (Greenberg, Bell, Guan, & Yu, 2011; Higdon, Victoria, Delage, & McNulty, 2000–2014). DHFRIs displace folate from the enzyme and block its conversion to the active tetrahydrofolates (Lambie & Johnson, 1985). Antiepileptic drugs may impact the body's ability to use folate through enzymes in the folate metabolism pathway other than dihydrofolate reductase, by impairing the absorption of folate, or by increased folate degradation (Hernandez-Diaz, Werler, Walker, & Mitchell, 2000; van Gelder et al., 2010).

In previous analyses of deliveries during 1976–1998 data included in the Slone Birth Defects Study (BDS), exposure to DHFRIs and AEDs in early pregnancy was associated with increased risk for NTDs, oral clefts, urinary defects, and cardiac defects (Hernandez-Diaz et al., 2000; Hernandez-Diaz, Werler, Walker, & Mitchell, 2001). In these analyses, the association appeared to be modified by folic acid supplementation for DHFRI exposure but not AED exposure (Hernandez-Diaz et al., 2001). Analyses of data from other studies have shown similar results for AEDs (Meijer, De Walle, & Kerstjens-Frederikse, 2005; Werler et al., 2011), but conflicting results for risk associated with DHFRI exposure, with some studies finding increased risks (Crider et al., 2009; Czeizel, Rockenbauer, Sorensen, & Olsen, 2001; Matok et al., 2009), and others finding no association (Hansen et al., 2016; Meijer et al., 2005). The previous findings from the Slone BDS were conducted using data collected prior to the implementation of mandatory folic acid fortification of the grain supply in the United States and Canada in 1998 (Canada Gazette part II: regulatory impact analysis statement, SOR/98–550., 1998; FDA, 1996). We sought to re-examine the use of these medications in data collected in the post-fortification era in the Slone BDS.

2 | METHODS

The Slone BDS is a multi-center case-control study of risk factors for major malformations that collected data on deliveries from 1976 to 2015. Its methods have been described in detail previously (Louik, Lin, Werler, Hernández-Díaz, & Mitchell, 2007). Infants, fetuses, and stillbirths with any major structural malformations (cases) were identified at participating hospitals or in birth defect registries for parts of Massachusetts, areas surrounding Philadelphia, PA; San Diego, CA (starting in 2001), Toronto, Canada (through 2005), Nashville, TN (starting in 2012); and parts of New York State (starting in 2004). During the time period included in our analysis (1998–2015), live-born infants without malformations from the same catchment areas as cases were identified as controls.

Mothers of eligible cases and controls were invited to participate in an interview within 6 months of delivery. Participating mothers completed a computer-assisted telephone interview that asked about demographic factors, maternal characteristics and reproductive history, detailed data on illnesses and use of medication, vitamins, and supplements in the 2 months prior to the last menstrual period (LMP) through the end of pregnancy. The interview also asked mothers about dietary patterns in the 6 months prior to pregnancy. The study has been approved by the institutional review boards of Boston University Medical Center and relevant participating institutions.

2.1 | Outcome definitions

We included any case infant with a malformation code for anencephaly, spina bifida, or encephalocele in the NTD case group. We separately identified cases of cleft lip and/or cleft palate for the oral cleft case group. The urinary defect case group consisted of infants with any major malformation of the urinary system. We also created a subgroup of non-obstructive urinary malformations (e.g., renal agenesis, extra/horseshoe kidney, etc.), which excluded obstructive defects such as hydronephrosis. The cardiac defect malformation group consisted of any major cardiac malformation. We identified subgroups with conotruncal malformations, ventricular septal defects, or other cardiac abnormalities. For all case groups, we also identified isolated cases as those having no other major malformation, or whose only additional malformation was of the same category.

For all case groups, we excluded any case with chromosomal or syndrome abnormalities, conjoined twins, or those with amniotic band diagnoses. The oral cleft case group also excluded infants with Pierre-Robin syndrome. For all analyses, controls were infants without any major malformation. We excluded subjects whose birth hospital did not contribute at least one case and one control infant to the study.

2.2 | Exposure definition

We separately considered two major groups of folic acid antagonists, DHFRIs and AEDs. We considered the following drugs to be DHFRIs: trimethoprim, triamterene, sulfasalazine, methotrexate, and proguanil. We considered the following AED medications: valproic acid, phenobarbital, phenytoin, primidone, carbamazepine, mephobarbital, lamotrigine, gabapentin, topiramate, oxcarbazepine, zonisamide, levetiracetam, pregabalin, lacosamide,

and anticonvulsant not otherwise specified (NOS). For consistency with prior literature, lamotrigine is considered here as an AED, although it has also been shown to inhibit DHFR in vitro ("Lamictal (lamotrigine) [package insert] GlaxoSmithKline, Research Triangle Park, NC 27709," 2009). The third group of folate antagonists, bile acid sequestrants (colestipol, colesevelam, and cholestyramine) are primarily used to lower cholesterol levels and have a low prevalence of exposure in women of childbearing age; these were, therefore, not examined in this analysis. We included exposures to both single and multiple component medications (notably trimethoprim-sulfamethoxazole).

In analyses of NTDs, the etiologically relevant exposure window of interest was the 2 lunar months periconceptional window, from 28 days prior to LMP through 28 days post-LMP. In analyses of oral clefts, urinary defects, and cardiac malformations, the etiologically relevant exposure window of interest was the first trimester, defined as LMP through the third lunar month (84 days). In all analyses, the reference group was mothers who did not report exposure to these drugs at any time from 2 months prior to LMP through delivery.

2.3 | Folic acid intake

Total periconceptional folic acid intake and total first trimester folic acid intake were calculated by combining the average daily synthetic folic acid intake from fortified foods and from supplements. Naturally occurring food folate was included but discounted by 30% because of its lower bioavailability (Intakes, 1998). Diet information was ascertained using an adapted Willett food frequency questionnaire (FFQ) focused on the 6 months before pregnancy, to reflect diet in the earliest stages of gestation when pregnancy might not yet be recognized. After calculating an average daily exposure amount from the FFQ, we adjusted these values for total caloric intake using the residual method (Willett & Stampfer, 1986). We then added this value to the reported average daily intake from supplements. Women who reported extreme caloric intake (<500 or >4,000 kcal per day) were excluded (1% of subjects). Our primary folic acid intake variable was created by dichotomizing average daily exposure at the recommended intake level of greater than or equal to 400 μg per day, with an intake of at least 400 μg per day considered to be "adequate" for NTD prevention. Average daily FA intake was calculated separately for the periconceptional period for the NTD analysis, and for the first trimester intake for the analysis of clefts, urinary, and cardiac malformations. Women with missing FFQ data were included and categorized as having adequate folic acid intake if they reported vitamin supplementation greater than or equal to 400 μg per day (3% of subjects), or categorized as having a folic acid intake of less than 400 μg per day if they reported no vitamin intake (6% of subjects for periconceptional intake and 2% of subjects for the first trimester intake) because it is unlikely these women would achieve greater than or equal to 400 μg per day from diet alone (Tinker, Cogswell, Devine, & Berry, 2010). Women with missing FFQ data were excluded if reported folic acid intake from vitamin supplementation was less than 400 μg per day because they may or may not have reached 400 μg per day depending on the diet (1% of subjects for periconceptional intake and 5% for the first trimester intake). In addition, less than 1% of subjects who completed the FFQ with less than 400 μg per day were excluded due to incomplete or unknown supplement data.

2.4 | Statistical analyses

We present the distribution of demographic and maternal health characteristics for cases and controls and by reported folic acid antagonist exposure among controls. We assessed maternal age, race/ethnicity, education, study center, pre-pregnancy body mass index (BMI), LMP year, trimester of prenatal care initiation, family history of birth defects, gravidity, parity, interpregnancy interval, pregnancy planning, alcohol intake, and smoking during pregnancy. We also sought to consider potential confounding by indication for use of folic acid antagonist medications. Although the DHFRIs have varied indications for use, the most commonly used of these medications is trimethoprim, an antibiotic used primarily in combination with sulfamethoxazole to treat urinary tract infections (UTIs). We, therefore, identified all mothers with any reported infection in the time period of interest (periconceptional or first trimester), and those who reported UTIs specifically. We also identified mothers who reported epilepsy, convulsions, or seizures. Because many of the newer AEDs are also used for the treatment of psychological conditions, we also considered the use of other psychoactive medications in the time period of interest, defined as any antidepressant, selective serotonin reuptake inhibitor (SSRI), benzodiazepine, barbiturate, phenothiazine, or atypical antipsychotic, to identify women with psychological conditions.

We calculated crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) using logistic regression. Variables were chosen for inclusion in adjusted models based on association with both exposure and outcome. We stratified all results by folic acid intake in order to examine its role as a potential effect modifier. All analyses were carried out in SAS 9.4.

3 | RESULTS

A total of 10,236 controls, 405 NTD cases, 1,286 oral cleft cases, 2,478 urinary defect cases, and 6,127 cardiac defect cases were available for analysis. We excluded subjects whose mothers reported folic acid antagonist exposure only outside of the relevant exposure window: 42 and 31 controls (periconceptional and first trimester, respectively); among cases, we excluded 3 NTDs, 2 oral clefts, 6 urinary defects, and 32 cardiac defects. Table 1 displays the characteristics of cases and controls. In general, compared to mothers of controls, mothers of cases were more likely to have lower education, be non-Hispanic Black or Hispanic, be overweight or obese, have their first prenatal visit after the first trimester, have a family history of birth defects, be non-drinkers during pregnancy, smoke during pregnancy, and have pre-existing diabetes. Mothers of NTD cases were more likely to report an infection during the periconceptional period than control mothers, but no difference was observed specifically for UTIs. Conversely, the prevalence of any infection during the first trimester was similar for mothers of controls and mothers of cases with oral clefts, urinary defects, and cardiac defects, but UTIs during the first trimester were more common among mothers of infants in these case groups than controls. Mothers of NTD and oral cleft cases were more like to have epilepsy, convulsions, and/or seizures than mothers of controls. Mothers of NTD cases were less likely to have adequate periconceptional folic acid intake than mothers of controls.

Table 2 displays characteristics of the folic acid antagonist exposed and unexposed, among controls. Among controls, periconceptional folic acid antagonist exposure was more common for mothers who did not have a college education, was non-Hispanic White, was underweight or obese, had their first prenatal visit in the first trimester, was primiparous, had an unintended pregnancy, did not drink during pregnancy, and smoked during pregnancy. Control mothers who reported folic acid antagonist exposure were more likely than unexposed control mothers to have an infection during the periconceptional period (particularly UTIs), use other antibiotics, have epilepsy, convulsions, and/or seizures, have other psychological illness in the periconceptional period, use an SSRI in the periconceptional period, have gestational diabetes, and had FA intake 400 mcg.

Periconceptional use of DHFRIs was rare; 0.2% of control mothers ($n = 16$) and no NTD case mothers were exposed (Table 3). Periconceptional AED exposure was reported in 1.7% of NTD case mothers ($n = 7$) and 0.5% of control mothers ($n = 53$). First trimester DHFRI exposure was similar among mothers of oral cleft cases ($n = 3$, 0.2%) and mothers of controls ($n = 26$, 0.3%). First trimester exposure to AEDs was more common among mothers of oral cleft cases ($n = 15$, 1.2%) than mothers of controls ($n = 53$, 0.5%). We observed higher exposure prevalence among mothers of urinary defect cases for both DHFRIs ($n = 9$, 0.4%) and AEDs ($n = 21$, 0.9%) than mothers of controls. First trimester DHFRI use was slightly less common among mothers of cardiac cases ($n = 10$, 0.2%) than among mothers of controls; AED exposures were more common in cardiac cases ($n = 49$, 0.8%). Exposure to specific medications is described in Supplementary Table 1.

Results from adjusted models were similar to those from the crude models; crude results are presented in Table 3 to allow more direct comparison with the results stratified by FA exposure. The OR for periconceptional AED exposure was 3.4 (95% CI: 1.5, 7.5) among those with NTD compared to those without NTD. The OR for AEDs was larger for mothers with sufficient FA intake compared to mothers with lower intake, although estimates were unstable due to small numbers.

The OR for DHFRI exposure and oral clefts was 0.9 (95% CI: 0.2, 3.0) (Table 3). The OR for any AED exposure was 2.3 (95% CI: 1.3, 4.0). This association was driven by an elevated OR for topiramate (OR: 5.8; 95% CI: 2.3, 14.5) [data not shown]. After stratification by folic acid intake, there were zero exposed cases with low intake, and AED risk remained elevated among those with the adequate intake (OR: 2.6; 95% CI: 1.4, 4.8).

Observed risk estimates for urinary defects were similar for both drug groups (OR: 1.4; 95% CI: 0.7, 3.1 for DHFRIs, and OR: 1.6; 95% CI: 1.0, 2.7 for AEDs). Data were insufficient to assess differences by FA intake; however, among women with adequate FA intake, compared to the overall risk estimates, OR estimates were similar for DHFRI (OR: 1.3; 95% CI: 0.6, 3.1) and slightly elevated for AEDs (OR = 1.8; 95% CI: 1.0, 3.1). In a sub-analysis limited to non-obstructive urinary malformations (Supplementary Table 2), OR estimates were slightly increased. The OR was 1.8 (95% CI: 0.8, 4.2) for DHFRI and 1.9 (95% CI: 1.1, 3.4) for AED. The DHFRI estimate was driven by exposure to the blood pressure drug triamterene (OR: 3.1; 95% CI: 0.5, 18.4). This association was most pronounced among the

934 cases with isolated non-obstructive urinary malformations, where three were exposed (OR: 8.3; [95% CI: 1.2, 48.9]) (data not shown).

Among cardiac defects, we observed an OR point estimate below the null for DHFRI exposure (0.6; 95% CI: 0.3, 1.3) and above the null for AED exposure (1.6; 95% CI: 1.1, 2.3) (Table 3). The most commonly reported folic acid antagonist among mothers of cases with cardiac defects was lamotrigine ($n = 14$, 0.2%, OR: 3.4; 95% CI: 1.4, 8.3). Among the specific cardiac defects, we observed the highest risk for lamotrigine and conotruncal defects: OR of 9.4 (95% CI: 3.3, 26.9) ($n = 7$ exposed cases, data not shown), which largely accounted for the association with cardiac defects overall (among non-conotruncal defects the OR was 2.0 [95% CI: 0.7, 5.8]).

3.1 | Additional analyses

Because the increased risk for conotruncal malformations among women who used lamotrigine was unexpected, we assessed the indication among women who reported its use. We found that among the seven lamotrigine-exposed conotruncal cases, six (86%) were for non-epilepsy indications (two for bipolar disorder, four for depression). The subject reporting use for epilepsy was additionally exposed to carbamazepine in the first trimester. Similarly, among controls, six of seven (86%) were for non-epilepsy indications; one subject reported polytherapy for epilepsy (primidone). The seven lamotrigine-exposed conotruncal cases averaged 67 days of exposure during the first trimester, with five of seven (71%) exposed throughout the first trimester. The corresponding numbers for controls were an average of 47 days of exposure and two of seven (28%) exposed throughout the first trimester.

We also addressed confounding by indication by further stratifying AED exposures by indication for use: epilepsy versus non-epilepsy. We had limited power to examine these associations, but report similar ORs for AED exposure and each of the four defect groups examined between those with epilepsy and non-epilepsy indications, with largely overlapping confidence intervals (see Supplementary Table 3). The numbers were too small to evaluate the indication for use of DHFR inhibitors.

4 | DISCUSSION

Similar to our previous studies (Hernandez-Diaz et al., 2000, 2001), we found an association between AED use and NTDs that was not attenuated by adequate FA intake. In contrast, we found DHFRI exposure was not associated with increased risks of NTDs, oral clefts, and cardiac defects, although we observed a slightly elevated association for urinary defects; we observed no clear evidence of FA effect modification. Our null results for DHFRI use may reflect higher population-level folate intake through food fortification that accommodates the greater demands for folate in women taking DHFRI. The relationship between folic acid intake and AED exposure deserves further investigation.

The present analysis was designed to update the initial Slone BDS analyses of folic acid antagonists with more recent data. However, we made several refinements to the earlier approach: the previous analysis used infants with other major malformations as controls,

whereas we were able to use control infants without malformations. In the original analysis of NTDs, the exposure window was the first 2 lunar months of pregnancy; we have instead used the periconceptional window of 1 lunar month prior to through 1 lunar month after LMP. Similarly, in the original analysis of oral clefts, urinary defects, and cardiac defects, second and third lunar month exposures were considered; we expanded the window to also include exposures in the first lunar month. Finally, the original analyses lacked detail on folic acid intake specifically, and instead used multivitamin exposure as a proxy for folic acid supplementation. The present analysis examined folic acid intake specifically through both supplementation and diet.

The main difference between the results of the current and previous analyses is the lack of an increased risk for malformations after DHFRI exposure. Following our initial observations in this population (Hernandez-Diaz et al., 2000, 2001), increased risks were also observed in Israel (Matok et al., 2009) and Hungary (Czeizel et al., 2001). The National Birth Defects Prevention Study, another large U.S. case-control study, found increased risks of anencephaly and specific cardiac malformations for sulfonamide exposure, largely related to exposure to trimethoprim/sulfamethoxazole, although a null association for oral clefts reported in that study is consistent with the findings reported here (Crider et al., 2009). A Dutch study found no association (Meijer et al., 2005), and a recent U.S. study found no evidence of increased risk for trimethoprim-sulfonamide exposure and cleft lip/palate, cardiovascular, or urinary system defects (Hansen et al., 2016). Although we saw a somewhat elevated point estimate for urinary malformations (1.6 [1.0, 2.7]), we observed a null association for oral clefts and a suggestion of a protective association for cardiac malformations. The analysis of NTDs was limited by a small sample size with no DHFRI exposed cases.

AED use was associated with increased risk for NTDs, clefts, urinary defects, and cardiac malformations in this dataset, with no apparent modifying effect of adequate folic acid intake. For three of the four defects considered, crude ORs were somewhat higher among those with adequate intake, although CIs were wide. This lack of decreased risk among those with adequate folic acid intake has been reported in other studies (Hill, Wlodarczyk, Palacios, & Finnell, 2010; Morrow et al., 2009; Vajda et al., 2019). We observed slightly lower risk estimates for AEDs than have been reported previously, perhaps reflecting a move away from AEDs with known teratogenic potential, especially valproate, for women during pregnancy. The finding of an increased risk for oral clefts among topiramate-exposed pregnancies has been reported previously, including in an analysis of BDS data (Alsaad, Chaudhry, & Koren, 2015; Margulis et al., 2012; Mines et al., 2014). The association between AEDs and cardiac malformations was driven by an elevated risk estimate for lamotrigine and conotruncal cardiac malformations. Previous research has found that drug to be relatively safe in comparison to other AEDs (Hernández-Díaz et al., 2012; Tomson et al., 2018; Vajda et al., 2010; Weston et al., 2016). The only suggestion of increased risk in prior studies comes from the International Lamotrigine Pregnancy Registry, which noted three cases of transposition of the great arteries (a conotruncal anomaly) among 1,817 infants exposed to lamotrigine monotherapy in the first trimester, a higher prevalence than was observed in a population-based reference (Cunnington et al., 2011). Other studies have not observed associations with cardiac defects overall (Dolk et al., 2016; Hernández-Díaz

et al., 2012; Moløgaard-Nielsen & Hviid, 2011; Veiby, Daltveit, Engelsen, & Gilhus, 2014; Werler et al., 2011), or conotruncal malformations specifically (Dolk et al., 2016).

The exposures to lamotrigine in this dataset are potentially confounded by exposure to other AEDs and other psychological illnesses and their treatment. These factors are known to complicate the potential risk profile for a given drug (Holmes, Mittendorf, Shen, Smith, & Hernandez-Diaz, 2011; Tomson et al., 2018). However, it is also notable that lamotrigine has been reported to weakly inhibit DHFR in vitro ("Lamictal (lamotrigine) [package insert] GlaxoSmithKline, Research Triangle Park, NC 27709," 2009). We observed a longer duration of use in early pregnancy among conotruncal cases compared to controls. That controls were more likely than cases to stop treatment may point to an effect of the drug by increasing the probability of exposure at a critical time in gestation, or to severity of the underlying condition being treated. These findings, coupled with the fact that the lamotrigine-exposed cases were most commonly the more serious conotruncal defects such as Tetralogy of Fallot or coarctation of the aorta, warrant further study, especially in the context of use for indications other than epilepsy.

The study has a number of strengths. We were able to define folic acid intake more precisely than previous analyses, while also accounting for various potential confounders that may not be identified in other datasets. Self-report of medication intake also allowed for characterization of patterns and timing of exposures that are not possible with administrative data because women reported directly when they took medication, rather than having to rely on the information on prescriptions or fill dates, which may not capture whether prescriptions are filled and do not capture whether filled prescriptions are taken as prescribed.

The strength of self-report may also represent a limitation, as medication use may be potentially subject to recall error; however, such errors in the recall are unlikely to account for the variations in findings for the specific exposures and outcomes studied. There were also small numbers for many of the associations we assessed, leading to unstable estimates. In addition, due to the small sample size, we were unable to control for some potential confounders without further decreasing the precision of estimates.

Overall, this study found little evidence of risk for the specific birth defects we assessed for DHFRI exposure in early pregnancy (based on limited power), but elevated risk estimates for AEDs, with no evidence of protection among those subjects exposed to adequate folic acid. Compared to findings from earlier reports of elevated risks for DHFRI exposure from pre-fortification studies, it is possible that elevated blood serum folate levels confer protection against the potential risk of those drugs to the fetus in early pregnancy. On the other hand, the lack of evidence for a protective effect of adequate folic acid intake after AED exposure, (with a sometimes slightly increased risk in this group) points to a potential different mechanism. The elevated risk estimates for certain specific drugs and specific malformations (topiramate and oral clefts, lamotrigine, and conotruncal heart defects), highlight the importance of continued research in this field.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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TABLE 1

Characteristics of cases and controls, Slone Birth Defects Study (1998–2015)

Characteristic	Controls (N = 10,209)	NTD (N = 402)	Oral Cleft (N = 1,284)	Urinary (N = 2,472)	Cardiac (N = 6,095)
Maternal age (years)					
<20	719 (7.1%)	32 (8.0%)	96 (7.5%)	157 (6.4%)	450 (7.4%)
20–24	1,517 (14.9%)	70 (17.4%)	221 (17.2%)	416 (16.9%)	1,007 (16.5%)
25–29	2,734 (26.8%)	126 (31.3%)	353 (27.5%)	663 (26.9%)	1,632 (26.8%)
30–34	3,361 (33.0%)	113 (28.1%)	406 (31.7%)	749 (30.3%)	1,916 (31.4%)
35–40	1,594 (15.7%)	52 (12.9%)	179 (14.0%)	407 (16.5%)	902 (14.8%)
40+	259 (2.5%)	9 (2.2%)	27 (2.1%)	76 (3.1%)	186 (3.1%)
Maternal education					
<High school	944 (9.3%)	61 (15.2%)	167 (13.0%)	294 (11.9%)	849 (14.0%)
High school	1,942 (19.0%)	97 (24.1%)	295 (23.0%)	528 (21.4%)	1,345 (22.1%)
>High school	7,312 (71.7%)	244 (60.7%)	820 (64.0%)	1,647 (66.7%)	3,891 (63.9%)
Maternal race/ethnicity					
Non-Hispanic White	6,722 (66.0%)	223 (55.5%)	807 (63.1%)	1,474 (59.8%)	3,558 (58.5%)
Non-Hispanic Black	854 (8.4%)	46 (11.4%)	106 (8.3%)	223 (9.1%)	568 (9.3%)
Hispanic	1,724 (16.9%)	94 (23.4%)	230 (18.0%)	540 (21.9%)	1,380 (22.7%)
Other	891 (8.7%)	39 (9.7%)	135 (10.6%)	226 (9.2%)	578 (9.5%)
Study center					
Boston	4,958 (48.6%)	71 (17.7%)	242 (18.8%)	538 (21.8%)	1,475 (24.2%)
Philadelphia	1,896 (18.6%)	98 (24.4%)	425 (33.1%)	592 (23.9%)	1,561 (25.6%)
Toronto	644 (6.3%)	93 (23.1%)	216 (16.8%)	222 (9.0%)	660 (10.8%)
San Diego	1,631 (16.0%)	70 (17.4%)	205 (16.0%)	614 (24.8%)	1,413 (23.2%)
New York	966 (9.5%)	57 (14.2%)	183 (14.3%)	463 (18.7%)	916 (15.0%)
Nashville	114 (1.1%)	13 (3.2%)	13 (1.0%)	43 (1.7%)	70 (1.1%)
Pre-pregnancy BMI					
Underweight (<18.5 kg/m^2)	426 (4.3%)	17 (4.4%)	57 (4.6%)	113 (4.7%)	229 (3.9%)
Normal weight (18.5–24.9 kg/m^2)	6,057 (60.9%)	195 (50.5%)	677 (54.2%)	1,313 (55.1%)	3,214 (54.7%)

Characteristic	Controls (N = 10,209)	NTD (N = 402)	Oral Cleft (N = 1,284)	Urinary (N = 2,472)	Cardiac (N = 6,095)
Overweight (25.0–24.9 kg/m²)	2,122 (21.3%)	96 (24.9%)	296 (23.7%)	567 (23.8%)	1,329 (22.6%)
Obese (30.0 kg/m²)	1,336 (13.4%)	78 (20.2%)	218 (17.5%)	391 (16.4%)	1,102 (18.8%)
LMP year					
1997–2002	3,439 (33.7%)	125 (31.1%)	279 (21.7%)	484 (19.6%)	1,431 (23.5%)
2003–2008	3,229 (31.6%)	147 (36.6%)	571 (44.5%)	864 (35.0%)	1,957 (32.1%)
2009–2014	3,541 (34.7%)	130 (32.3%)	434 (33.8%)	1,124 (45.5%)	2,707 (44.4%)
First prenatal visit in T1					
No	415 (4.1%)	35 (8.8%)	79 (6.2%)	151 (6.1%)	369 (6.1%)
Yes	9,753 (95.9%)	364 (91.2%)	1,197 (93.8%)	2,316 (93.9%)	5,686 (93.9%)
Family history of birth defects (first degree)					
No	9,193 (90.0%)	360 (89.6%)	1,070 (83.3%)	2,162 (87.5%)	5,227 (85.8%)
Yes	1,016 (10.0%)	42 (10.4%)	214 (16.7%)	310 (12.5%)	868 (14.2%)
Parity					
Primiparous	4,317 (42.3%)	161 (43.9%)	549 (43.0%)	999 (40.6%)	2,605 (42.8%)
Multiparous	5,891 (57.7%)	206 (56.1%)	728 (57.0%)	1,459 (59.4%)	3,477 (57.2%)
Pregnancy planned					
No	3,725 (36.5%)	156 (38.8%)	504 (39.3%)	916 (37.1%)	2,333 (38.3%)
Yes	6,469 (63.5%)	246 (61.2%)	778 (60.7%)	1,553 (62.9%)	3,755 (61.7%)
Any drinking during pregnancy					
None	4,995 (49.0%)	226 (56.2%)	677 (52.8%)	1,329 (53.8%)	3,345 (54.9%)
Any	5,198 (51.0%)	176 (43.8%)	606 (47.2%)	1,139 (46.2%)	2,745 (45.1%)
Any alcohol in T1					
None	8,502 (83.8%)	349 (87.3%)	1,077 (84.3%)	2,124 (86.4%)	5,196 (85.6%)
Any	1,648 (16.2%)	51 (12.8%)	201 (15.7%)	334 (13.6%)	876 (14.4%)
Smoking					
Never	7,092 (69.5%)	278 (69.2%)	873 (68.1%)	1,777 (71.9%)	4,344 (71.3%)
Before pregnancy only	1,608 (15.8%)	56 (13.9%)	166 (13.0%)	297 (12.0%)	738 (12.1%)

Characteristic	Controls (N = 10,209)	NTD (N = 402)	Oral Cleft (N = 1,284)	Urinary (N = 2,472)	Cardiac (N = 6,095)
Any during pregnancy	1,505 (14.7%)	68 (16.9%)	242 (18.9%)	398 (16.1%)	1,009 (16.6%)
Any infection in the periconceptional period					
No	8,453 (82.8%)	306 (76.1%)			
Yes	1,756 (17.2%)	96 (23.9%)			
Any infection in T1					
No	5,379 (52.7%)		631 (49.1%)	1,273 (51.5%)	3,139 (51.5%)
Yes	4,830 (47.3%)		653 (50.9%)	1,199 (48.5%)	2,956 (48.5%)
Any UTI in periconceptional period					
No	10,038 (98.3%)	394 (98.0%)			
Yes	171 (1.7%)	8 (2.0%)			
Any UTI in T1					
No	9,536 (93.4%)		1,179 (91.8%)	2,257 (91.3%)	5,600 (91.9%)
Yes	673 (6.6%)		105 (8.2%)	215 (8.7%)	495 (8.1%)
Any non-folic acid antagonist antibiotic exposure in the periconceptional period					
No	7,555 (96.1%)	291 (94.8%)			
Yes	304 (3.9%)	16 (5.2%)			
Any non-folic acid antagonist antibiotic exposure in T1					
No	7,555 (90.7%)		936 (89.1%)	1,780 (89.9%)	4,326 (89.0%)
Yes	776 (9.3%)		115 (10.9%)	199 (10.1%)	536 (11.0%)
Any reported epilepsy/convulsions/seizures					
No	10,163 (99.5%)	397 (98.8%)	1,271 (99.0%)	2,457 (99.4%)	6,057 (99.4%)
Yes	46 (0.5%)	5 (1.2%)	13 (1.0%)	15 (0.6%)	38 (0.6%)
Any psychological illness in the periconceptional period					
No	9,705 (95.1%)	384 (95.5%)			
Yes	504 (4.9%)	18 (4.5%)			
Any psychological illness in T1					
No	9,694 (95.0%)		1,204 (93.8%)	2,341 (94.7%)	5,679 (93.2%)

Characteristic	Controls (N = 10,209)	NTD (N = 402)	Oral Cleft (N = 1,284)	Urinary (N = 2,472)	Cardiac (N = 6,095)
Yes	515 (5.0%)		80 (6.2%)	131 (5.3%)	416 (6.8%)
Any SSRI exposure in the periconceptional period					
No	9,798 (96.8%)	387 (97.0%)			
Yes	323 (3.2%)	12 (3.0%)			
Any SSRI exposure in T1					
No	9,798 (96.7%)		1,212 (95.9%)	2,377 (97.0%)	5,791 (95.8%)
Yes	336 (3.3%)		52 (4.1%)	73 (3.0%)	257 (4.2%)
Diabetes					
Never	9,634 (94.4%)	365 (90.8%)	1,177 (91.7%)	2,182 (88.3%)	5,330 (87.4%)
Pre-existing	59 (0.6%)	10 (2.5%)	24 (1.9%)	62 (2.5%)	238 (3.9%)
Gestational	516 (5.1%)	27 (6.7%)	83 (6.5%)	228 (9.2%)	527 (8.6%)
Periconceptional folic acid intake					
Inadequate (<400 mcg)	4,616 (45.2%)	204 (50.7%)			
Adequate (400 mcg+)	5,342 (52.3%)	183 (45.5%)			
Missing	251 (2.5%)	15 (3.7%)			
T1 folic acid intake					
Inadequate (<400 mcg)	1,647 (16.1%)		238 (18.5%)	432 (17.5%)	1,065 (17.5%)
Adequate (400 mcg+)	7,915 (77.5%)		951 (74.1%)	1,829 (74.0%)	4,485 (73.6%)
Missing	647 (6.3%)		95 (7.4%)	211 (8.5%)	545 (8.9%)

Abbreviations: BMI, body mass index; LMP, last menstrual period; NTD, neural tube defect; periconceptional, 1 lunar month pre-LMP through lunar month 1; SSRI, selective serotonin reuptake inhibitor; T1, first trimester (lunar months 1–3); UTI, urinary tract infection.

TABLE 2

Characteristics of folic acid antagonist exposures among controls, Slone Birth Defects Study (1998–2015)

Characteristic	Unexposed throughout pregnancy (N = 10,126)	Any periconceptional polic acid antagonist exposure (N = 68)	Any first trimester folic acid antagonist exposure (N = 79)
Maternal age (years)			
<20	714 (7.1%)	4 (5.9%)	5 (6.3%)
20–24	1,497 (14.8%)	18 (26.5%)	18 (22.8%)
25–29	2,713 (26.9%)	15 (22.1%)	20 (25.3%)
30–34	3,339 (33.1%)	18 (26.5%)	21 (26.6%)
35–40	1,580 (15.6%)	12 (17.6%)	14 (17.7%)
40+	258 (2.6%)	1 (1.5%)	1 (1.3%)
Maternal education			
<High school	937 (9.3%)	7 (10.3%)	6 (7.6%)
High school	1,918 (19.0%)	21 (30.9%)	24 (30.4%)
>High school	7,260 (71.8%)	40 (58.8%)	49 (62.0%)
Maternal race/ethnicity			
Non-Hispanic White	6,661 (65.9%)	52 (76.5%)	59 (74.7%)
Non-Hispanic Black	846 (8.4%)	6 (8.8%)	6 (7.6%)
Hispanic	1,713 (16.9%)	9 (13.2%)	11 (13.9%)
Other	888 (8.8%)	1 (1.5%)	3 (3.8%)
Study center			
Boston	4,917 (48.6%)	35 (51.5%)	38 (48.1%)
Philadelphia	1,882 (18.6%)	11 (16.2%)	14 (17.7%)
Toronto	638 (6.3%)	5 (7.4%)	5 (6.3%)
San Diego	1,622 (16.0%)	6 (8.8%)	9 (11.4%)
New York	954 (9.4%)	10 (14.7%)	12 (15.2%)
Nashville	113 (1.1%)	1 (1.5%)	1 (1.3%)
Pre-pregnancy BMI			
Underweight (<18.5 kg/m²)	420 (4.3%)	6 (9.0%)	6 (7.7%)
Normal weight (18.5–24.9 kg/m²)	6,017 (61.0%)	31 (46.3%)	38 (48.7%)

Characteristic	Unexposed throughout pregnancy (N = 10,126)	Any periconceptional folic acid antagonist exposure (N = 68)	Any first trimester folic acid antagonist exposure (N = 79)
Overweight (25.0–24.9 kg/m^2)	2,104 (21.3%)	13 (19.4%)	17 (21.8%)
Obese (30.0 kg/m^2)	1,318 (13.4%)	17 (25.4%)	17 (21.8%)
LMP year			
1997–2002	3,409 (33.7%)	24 (35.3%)	26 (32.9%)
2003–2008	3,204 (31.6%)	21 (30.9%)	25 (31.6%)
2009–2014	3,513 (34.7%)	23 (33.8%)	28 (35.4%)
First prenatal visit in T1			
No	414 (4.1%)	1 (1.5%)	1 (1.3%)
Yes	9,671 (95.9%)	67 (98.5%)	78 (98.7%)
Family history of birth defects (first degree)			
No	9,121 (90.1%)	59 (86.8%)	69 (87.3%)
Yes	1,005 (9.9%)	9 (13.2%)	10 (12.7%)
Parity			
Primiparous	4,271 (42.2%)	41 (60.3%)	45 (57.0%)
Multiparous	5,854 (57.8%)	27 (39.7%)	34 (43.0%)
Pregnancy planned			
No	3,682 (36.4%)	37 (54.4%)	41 (51.9%)
Yes	6,429 (63.6%)	31 (45.6%)	38 (48.1%)
Any drinking during pregnancy			
None	4,946 (48.9%)	41 (60.3%)	48 (60.8%)
Any	5,164 (51.1%)	27 (39.7%)	31 (39.2%)
Any alcohol in T1			
None	8,429 (83.7%)	61 (89.7%)	71 (89.9%)
Any	1,638 (16.3%)	7 (10.3%)	8 (10.1%)
Smoking			
Never	7,045 (69.6%)	38 (55.9%)	47 (59.5%)
Before pregnancy only	1,596 (15.8%)	11 (16.2%)	10 (12.7%)

Characteristic	Unexposed throughout pregnancy (N = 10,126)	Any periconceptional folic acid antagonist exposure (N = 68)	Any first trimester folic acid antagonist exposure (N = 79)
Any during pregnancy	1,481 (14.6%)	19 (27.9%)	22 (27.8%)
Any infection in the periconceptional period			
No	8,400 (83.0%)	22 (32.4%)	
Yes	1,726 (17.0%)	46 (67.6%)	
Any infection in T1			
No	5,355 (52.9%)		51 (64.6%)
Yes	4,771 (47.1%)		28 (35.4%)
Any UTI in periconceptional period			
No	9,967 (98.4%)	49 (72.1%)	
Yes	159 (1.6%)	19 (27.9%)	
Any UTI in T1			
No	9,482 (93.6%)		67 (84.8%)
Yes	644 (6.4%)		12 (15.2%)
Any non-folic acid antagonist antibiotic exposure in the periconceptional period			
No	7,509 (96.2%)	37 (82.2%)	
Yes	297 (3.8%)	8 (17.8%)	
Any non-folic acid antagonist antibiotic exposure in T1			
No	7,509 (90.8%)		43 (87.8%)
Yes	764 (9.2%)		6 (12.2%)
Any reported epilepsy/convulsions/seizures			
No	10,107 (99.8%)	42 (61.8%)	52 (65.8%)
Yes	19 (0.2%)	26 (38.2%)	27 (34.2%)
Any psychological illness in the periconceptional period			
No	9,641 (95.2%)	62 (78.5%)	
Yes	485 (4.8%)	17 (21.5%)	
Any psychological illness in T1			

Characteristic	Unexposed throughout pregnancy (N = 10,126)	Any periconceptional folic acid antagonist exposure (N = 68)	Any first trimester folic acid antagonist exposure (N = 79)
No	9,630 (95.1%)	58 (86.6%)	51 (75.0%)
Yes	496 (4.9%)	9 (13.4%)	17 (25.0%)
Any SSRI exposure in the periconceptional period			
No	9,726 (96.9%)	58 (86.6%)	51 (75.0%)
Yes	313 (3.1%)	9 (13.4%)	17 (25.0%)
Any SSRI exposure in T1			
No	9,726 (96.8%)	63 (92.6%)	69 (88.5%)
Yes	326 (3.2%)	0 (0.0%)	9 (11.5%)
Diabetes			
Never	9,556 (94.4%)	63 (92.6%)	75 (94.9%)
Pre-existing	59 (0.6%)	0 (0.0%)	0 (0.0%)
Gestational	511 (5.0%)	5 (7.4%)	4 (5.1%)
Periconceptional folic acid intake			
Inadequate (<400 mcg)	4,576 (45.2%)	11 (16.2%)	
Adequate (400 mcg+)	5,299 (52.3%)	55 (80.9%)	
Missing	251 (2.5%)	2 (2.9%)	
T1 folic acid intake			
Inadequate (<400 mcg)	1,634 (16.1%)		38 (48.1%)
Adequate (400 mcg+)	7,848 (77.5%)		41 (51.9%)
Missing	644 (6.4%)		0 (0.0%)

Abbreviations: BMI, body mass index; LMP, last menstrual period; periconceptional, 1 lunar month pre-LMP through lunar month 1); SSRI, selective serotonin reuptake inhibitor; T1, first trimester (lunar months 1–3); UTI, urinary tract infection.

Note: Folic acid antagonists include dihydrofolate reductase inhibitors: trimethoprim, triamterene, sulfasalazine, methotrexate, proguanil; anti-epileptic drugs: valproic acid, carbamazepine, phenytoin, primidone, phenobarbital, topiramate, lamotrigine, gabapentin, levetiracetam, oxcarbazepine, pregabalin, zonisamide, an anti-epileptic drug not otherwise specified.

TABLE 3

Folic acid antagonist exposure and risk for neural tube defects, oral clefts, urinary malformations, and cardiac malformations– stratified by folic acid intake, Slone Birth Defects Study, 1998–2015

| | Periconceptional exposure | | | First trimester exposure | | | | | | |
	Controls (N = 10,194[a])	NTDs (N = 402)	Crude OR (95% CI)	Controls (N = 10,205)	Oral Cleft (N = 1,284)	Crude OR (95% CI)	Urinary (N = 2,472)	Crude OR (95% CI)	Cardiac (N = 6,095)	Crude OR (95% CI)
Folic acid antagonist exposure										
None	10,126 (99.3%)	395 (98.3%)	REF	10,126 (99.2%)	1,266 (98.6%)	REF	2,441 (98.7%)	REF	6,036 (99.0%)	REF
DHFR inhibitor	16 (0.2%)	0 (0%)	–	26 (0.3%)	3 (0.2%)	0.9 (0.2, 3.0)	9 (0.4%)	1.4 (0.7, 3.1)	10 (0.2%)	0.6 (0.3, 1.3)
Adequate FA intake (400 mcg+)[b]	9 (0.1%)	0 (0%)	–	23 (0.2%)	1 (<0.1%)	–	7 (0.3%)	1.3 (0.6, 3.1)	8 (0.1%)	0.6 (0.3, 1.4)
Inadequate FA intake (<400 mcg)	7 (0.1%)	0 (0%)	–	2 (<0.1%)	0 (0.0%)	–	1 (<0.1%)	–	2 (<0.1%)	1.5 (0.1, 21.4)
Any AED	53 (0.5%)	7 (1.7%)	3.4 (1.5, 7.5)	53 (0.5%)	15 (1.2%)	2.3 (1.3, 4.0)	21 (0.9%)	1.6 (1.0, 2.7)	49 (0.8%)	1.6 (1.1, 2.3)
Adequate FA intake (400 mcg+)[b]	24 (0.2%)	4 (1.0%)	4.9 (1.2, 14.6)	42 (0.4%)	13 (1.0%)	2.6 (1.4, 4.8)	17 (0.7%)	1.8 (1.0, 3.1)	39 (0.6%)	1.6 (1.1, 2.5)
Inadequate FA intake (<400 mcg)	29 (0.3%)	2 (0.5%)	1.6 (0.2, 6.2)	9 (0.1%)	0 (0.0%)	–	1 (<0.1%)	–	6 (0.1%)	1.0 (0.4, 2.9)

Abbreviations: AED, anti-epileptic drug; CI, confidence interval; DHFR, dihydrofolate reductase; FA, folic acid, NTD, neural tube defect; OR, odds ratio; periconceptional, 1 lunar month pre-LMP through first lunar month.

Note: DHFR Inhibitors: trimethoprim, triamterene, sulfasalazine, methotrexate, proguanil. AEDs: valproic acid, carbamazepine, phenytoin, primidone, phenobarbital, topiramate, lamotrigine, gabapentin, levetiracetam, oxcarbazepine, pregabalin, zonisamide, AED not otherwise specified.

[a] Column numbers may not sum to total. Three subjects were exposed to both a DHFR inhibitor and an AED (one control mother, one urinary malformation case mother, and one cardiac case mother). Four subjects were exposed to bile acid sequestrants and were not included in the analysis (one control mother in the first trimester, two urinary malformation case mothers, and one cardiac case mother).

[b] Folic acid intake subgroups may not sum to total. Subjects with missing folic acid intake data not shown.